1. PURPOSE

1.1. The purpose of this procedure is to describe the maintenance of HEK293TT cells.

2. SCOPE

2.1. This procedure applies to the Human Papillomavirus (HPV) Serology Laboratory located at the Advanced Technology Research Facility (ATRF), Room C2007.

2.2. This procedure applies to HEK293TT (293TT) cells that are used in the production of HPV L1 or L1/L2 virus-like particles (VLPs), HPV L1/L2 pseudovirions (PsV) and for cell-based assays.

3. REFERENCES

3.1. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance

3.2. HSL_EQ_002: Operation, Use and Maintenance of C02 Incubators

3.3. HSL_EQ_003: Use and Maintenance of the Thermo Fisher Sorvall Legend XTR Centrifuge in the HPV Serology Laboratory

3.4. HSL_EQ_006: Use and Maintenance of the Cellometer Auto 2000

3.5. HSL_EQ_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory

3.6. HSL_EQ_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory

3.7. HSL_EQ_009: Use and Maintenance of the Liquid Nitrogen Freezer

3.8. HSL_EQ_010: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath

3.9. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory

3.10. HSL_EQ_018: Use and Maintenance of an Inverted Microscope

3.11. HSL_EQ_022: Controlled-Rate Cell Freezing Using a CoolCell Device

3.12. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility

3.13. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory

3.14. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures

3.15. HSL_QS_022: Lot Number Assignment

4. RESPONSIBILITIES

4.1. The Research Associate, hereafter referred to as analyst, is responsible for reviewing and following this procedure.

4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

5. DEFINITIONS

Term	Definition
DMEM	Dulbecco's Modified Eagle's Medium
DPBS	Dulbecco's Phosphate Buffered Saline
FBS	Heat-inactivated Fetal Bovine Serum
SDS	Safety Data Sheets

6. REAGENTS, MATERIALS AND EQUIPMENT

6.1. Reagents

6.1.1. HEK293TT cells (stored in liquid nitrogen (LN_2))

6.1.2. DMEM (Life Technologies, Cat # 11965-126)

6.1.3. FBS (GE, Cat # SH30070.03HI)

6.1.4. MEM Nonessential amino acids (Life Technologies, Cat # 11140-050)

6.1.5. Glutamax-I (Life Technologies, Cat # 35050-061)

6.1.6. Hygromycin B 50 mg/mL (Life Technologies, Cat # 10687-010)

6.1.7. Trypsin-EDTA (0.05%), phenol red (Life Technologies, Cat # 25300-054)

6.1.8. Trypan blue 0.4% (Life Technologies, Cat # 15250-061)

6.1.9. DPBS (Life Technologies, Cat # 14190-235)

6.1.10. ViaStain AOPI Staining Solution (Nexcelom, Cat # CS1-0106-5mL)

6.1.11. Cavicide (Warehouse, Cat # 79300360)

6.1.12. Ster-ahol (VWR, Cat # 14003-358 or equivalent)

6.1.13. Clorox Bleach, Concentrated (Warehouse, Cat # 68100251 or equivalent)

6.2. Consumables

6.2.1. T-150 Flask (Thomas Scientific, Cat # 9381J33 or equivalent)

6.2.2. T-225 Flask (Thomas Scientific, Cat # 9381M60 or equivalent)

6.2.3. 5-layer Flask (VWR, Cat # 89204-478 or equivalent)

6.2.4. 8-layer CELLdisk (Greiner Bio-one, Cat # 678108 or equivalent)

6.2.5. 50 mL Sterile Conical Tubes (Warehouse, Cat # 66401493 or equivalent)

6.2.6. 5 mL Serological Pipettes (Warehouse, Cat # 66401365 or equivalent)

6.2.7. 10 mL Serological Pipettes (Warehouse, Cat # 66401370 or equivalent)

6.2.8. 25 mL Serological Pipettes (Warehouse, Cat # 66401361 or equivalent)

6.2.9. 50 mL Serological Pipettes (Warehouse, Cat # 66401363 or equivalent)

6.2.10. 1.5 mL Polypropylene Tube (cryovial) (VWR, Cat # 87003-296 or equivalent)

6.2.11. Alcohol-resistant laboratory marker (Warehouse, Cat # 66400058)

6.2.12. Nalgene 0.2 µm PES membrane 500 mL filter bottle (Thomas Scientific, Cat # 1234K58 or equivalent)

6.2.13. Nalgene 0.2 µm PES membrane 1000 mL filter bottle (Thomas Scientific, Cat # 1234K59 or equivalent)

6.2.14. Rainin Pipette Tips

6.2.15. Disposable Hemocytometer (Nexcelom, Cat # CP2-002)

6.2.16. Wypalls paper towels (Warehouse, Cat # 79300335 or equivalent)

6.3. Equipment

6.3.1. Hemocytometer (Improved Neubauer 0.1mm deep)

6.3.2. Inverted Light Microscope (Nikkon TMS and Nikkon LBOPHOT or equivalent)

6.3.3. Nexcel Bioscience Cellometer Auto 2000 Cell Viability Counter

6.3.4. Water Bath

6.3.5. Centrifuge

6.3.6. Rainin Pipettes

6.3.7. CoolCell Devices (VWR, Cat # 75779-724 or equivalent)

6.3.8. Serologic Pipettor

6.3.9. Class II Biosafety Cabinet (BSC)

6.3.10. CO_2 Incubator

6.3.11. LN_2 Freezer

6.3.12. -80°C Freezer

6.3.13. Refrigerator

7. HEALTH AND SAFETY CONSIDERATIONS

7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.

7.2. Refer to the respective SDS when working with any chemicals.

7.3. Refer to “HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility” regarding waste disposal processes at the ATRF.

7.4. All work should be performed inside a Class II Biosafety Cabinet (BSC).

7.5. All surfaces should be decontaminated with Cavicide before and after each experiment.

7.6. All contaminated BSL-2 level liquid waste must be decontaminated using 10% Clorox bleach (final concentration) with a minimum contact time of 30 minutes before sink disposal.

8. PROCEDURE PRINCIPLES

8.1. The cell culture lot number is assigned per "HSL_QS_022: Lot Number Assignment."

9. REAGENT PREPARATION

9.1. Thawing Media (293TT TM)

9.1.1. Refer to "HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory," Section 17.

9.2. Maintenance Media (293TT MM)

9.2.1. Refer to HSL_GL_006, Section 18.

9.3. Freezing Media (293TT FM)

9.3.1. Refer to HSL_GL_006, Section 19.

10. PREPARING A NEW CELL LINE FROM FROZEN CELLS

10.1. Prepare a 150 cm^2 (T-150) flask with 30 mL of Thawing Media (293TT TM) and store at room temperature. Label the flask with the cell culture lot number per step 8.1.

10.2. Remove one vial of 293TT cells from the liquid nitrogen freezer, and store on dry ice if not immediately processed at step 10.3.

Note: Update LN_2 vial inventory to maintain cell line vial tracking and select "Freezer Inventory Updated" on "HSL_LAB_001.02: 293TT Cell Thaw Form."

10.3. Immerse frozen vial in a 37°C water bath being careful to not immerse the cap of the tube, since water may inadvertently enter tube.

Note: Do not leave the vials in a water bath by placing them in a floater that holds tubes.

10.4. Swirl tube in water and continually inspect the tube to verify how much the cells have thawed.

10.5. Remove the tube from the water bath once cells are mostly thawed and only a small frozen pellet remains.

10.6. Dry off tube with paper towel. Spray a piece of paper towel with Ster-ahol and use it to wipe the tube. Dry tube with paper towel.

10.7. Place the thawed vial of cells inside the BSC.

Note: Once the thawing process has begun, the cells should not be left unattended. The process of thawing and plating the cells must be carried out without interruptions or delays.

10.8. Invert tube to mix then assess viability per "HSL_EQ_008: Use and Maintenance of the Cellometer Auto 2000" or "Attachment 1: Counting Cells with a Hemocytometer" for manual counting instructions.

10.9. Record the cell count and viability of the thawed cells on HSL_LAB_001.02. Continue with culturing the cells if the viability is ≥60%; otherwise, a new vial of cells needs to be thawed.

Note: Record cell count to the nearest tenth.

10.10. Pipette all the thawed cells directly into the flask with 293TT TM from step 10.1.

10.11. Incubate flask(s) in a 37°C, 5% CO_2 incubator.

10.12. After 72 hours, inspect cell culture for adherence.

10.13. Once the cells are adherent and confluency reaches 70%-95% (~every 3-4 days), split cells per Section 11. This will be "Passage 1" and will begin the cell passage count.

10.14. Record maintenance on "HSL_LAB_001.03: 293TT Cell Culture Maintenance Form."

11. MAINTAINING CELL CULTURE

11.1. 293TTs should be split when confluency reaches 70%-95% (approximately every 3-4 days) after adherence and should be maintained for use no more than 20 passages. Any deviations require approval by the Scientific Manager.

11.2. Remove the flasks from the 37°C, 5% CO_2 incubator and place into BSC.

11.3. Discard media from the flask.

11.3.1. Place the flask upright and tilt it so that media collects into a corner of the side of the flask which does not contain cells to minimize loss of cells.

11.3.2. Aspirate the media in the flask using a sterile serological pipette and discard into a waste container with bleach.

11.3.3. For the multi-layer flasks, decant the media into a waste container with bleach.

11.4. Using a sterile serological pipette, add sterile DPBS to each flask per Table 1. Do not dispense DPBS directly onto cells, as they will become detached.

Table 1: Volume of DPBS

Flask Type	DPBS (mL)
T150 and T225	5 - 15
5-Layer	20 - 30
8-Layer CELLdisk	40 - 60

11.5. Gently rinse cells with DPBS by slowly rotating the flask, so that DPBS washes over the cells. Rotate flask 3-5 times and then stand flask upright.

11.6. Discard DPBS into waste container.

11.6.1. Tilt flask so the DPBS collects into a corner of the flask where there are no cells adhering to flask surface.

11.6.2. Aspirate DPBS from the flask using a sterile serological pipette and discard into the waste container.

11.6.3. For the multi-layer flasks, decant into a waste container.

11.7. Wash with DPBS one additional time, if needed per steps 11.4 to 11.6.

11.8. Using a sterile serological pipette, add 0.05% Trypsin-EDTA into each flask per Table 2.

Table 2: Volume of Trypsin-EDTA

Flask Type	Trypsin-EDTA (mL)
T150 and T225	3 - 5
5-Layer	12 - 20
8-Layer CELLdisk	27 - 45

11.9. Gently rotate the flask to distribute the trypsin evenly over the cells.

11.10. Place the flask(s) into a 37°C, 5% CO_2 incubator for 3-5 minutes. Use a timer during this step to minimize time cells are exposed to the trypsin, since prolonged exposure is toxic to cells.

Note: Optimal incubation temperature for trypsin is 37°C. Analyst writes comment on HSL_LAB_001.03 if another incubation temperature used (e.g., Room Temperature).

11.11. After the incubation, take the flasks out and rock back and forth to detach the cells completely. Continue rocking the flask until all cells have detached; verify by using a microscope per “HSL_EQ_018: Use and Maintenance of an Inverted Microscope.”

Note: A gentle tap may be used to help cell detachment.

11.12. Place flasks into BSC. Using a sterile serological pipette, add 293TT MM to each flask per Table 3 and gently rinse the flask with the media.

Table 3: Volume of 293TT MM

Flask Type	293TT MM (mL)
T150 and T225	10
5-Layer	40
8-Layer CELLdisk	90

11.13. Using a sterile serological pipette, remove media and cells from the flask and transfer into an appropriately sized sterile bottle or tube. For multi-layer flasks, decant the cells.

11.14. Repeat step 11.12, if necessary.

11.15. Tighten cap on tube. Gently mix the cells by inverting the tube several times.

11.16. Count cells per HSL_EQ_008 or Attachment 1.

11.17. Capture cell counts and viability on HSL_LAB_001.03.

Note: Record cell count to the nearest tenth.

11.17.1. Viability for each count must be ≥ 80% for cell concentration to be used. If the viability fails, the cell count is not used to calculate cell concentration. Repeat steps 11.16 and 11.17 as needed; refer to Table 4.

11.17.2. The Percent Difference of passing Cell Concentration 1 and Cell Concentration 2 needs to be ≤ 25% for the count to be considered valid.

Note: Record Percent Difference as a whole number.

11.17.2.1. To calculate Percent Difference:

Where:
C1 = Count 1
C2 = Count 2
Average count = (C1+C2) ÷ 2

% Difference = $\left|\frac{C1-C2}{Average\ Count}\right| \times 100\%$

11.17.3. If the results are not ≤ 25%, repeat steps 11.16 and 11.17 as needed; refer to Table 4.

Table 4: Cell Count Process Steps

Process steps when % Viability passes for Counts 1 and 2.						
% Viability		Avg Cell Count*	% Difference*	% Viability		Avg Cell Count*
Count 1	Count 2			Count 3	Count 4	
P	P	(Count 1 + 2) ÷ 2	P			
P	P	(Count 1 + 2) ÷ 2	F	P	P	(Count 1 + 2 + 3 + 4) ÷ 4
P	P	(Count 1 + 2) ÷ 2	F	P	F	(Count 1 + 2 + 3) ÷ 3
P	P	(Count 1 + 2) ÷ 2	F	F	P	(Count 1 + 2 + 4) ÷ 3

Process steps when % Viability fails for Count 1 and/or 2.						
% Viability				Avg Cell Count*	% Difference*	Additional Actions
Count 1	Count 2	Count 3	Count 4			
P	F	P		(Count 1 + 3) ÷ 2	P	
P	F	F	P	(Count 1 + 4) ÷ 2	P	Alert Scientific Manager
P	F	P		(Count 1 + 3) ÷ 2	F	Perform Count 4, and average cell count is (Count 1 + 3 +4) ÷ 3
P	F	F	P	(Count 1 + 4) ÷ 2	F	Alert Scientific Manager, Scientific Manager will perform two counts.
F	F	P	P	(Count 3 + 4) ÷ 2	P	Alert Scientific Manager
F	F	F				Perform manual Trypan Blue exclusion count and discuss with Scientific Manager, Scientific Manager will perform two counts.
F	F	P	P	(Count 3 + 4) ÷ 2	F	Alert Scientific Manager, Scientific Manager will perform two counts.
F	P	P		(Count 2 + 3) ÷ 2	P	
F	P	F	P	(Count 2 + 4) ÷ 2	P	Alert Scientific Manager
F	P	P		(Count 2 + 3) ÷ 2	F	Perform Count 4, and average cell count is (Count 2 + 3 +4) ÷ 3

* Passing cell counts used in calculation only.

11.18. Inoculate daughter flasks per Table 5.

Table 5: Recommended Flask Inoculation (Expected Growth Rates)

Flask Type	3 Day Incubation (million cells)	4 Day Incubation (million cells)	Total Volume (mL)
T150	9 (± 2)	4 (+ 2)	25-35
T225	10 (± 2)	4.5 (+ 2.5)	45-55
5-Layer	40 (± 5)	18 (+ 7)	180-220
8-Layer CELLdisk	90 (± 10)	40 (+ 20)	420-480

Note: If culture is growing slower than expected, inoculate daughter flasks per Table 6.

Table 6: Recommended Flask Inoculation (Slower than Expected Growth Rates)

Flask Type	3 Day Incubation (million cells)	4 Day Incubation (million cells)	Total Volume (mL)
T150	12 -18	6 - 9	25-35
T225	14 - 20	7 - 10	45-55
5-Layer	50 - 100	25 - 50	180-220
8-Layer CELLdisk	120 - 200	60 - 100	420-480

11.18.1. To determine volume of cells to seed per flask, use the following calculation:

(Desired inoculation cell number ÷ concentration of cells [cells/mL]) = volume of cells to be added to flask

For example, $4x10^6$ cells/flask ÷ $1x10^6$ cells/mL = 4 mL of cells per flask

11.18.2. To determine the amount of media required per flask, subtract the volume of cells added to each flask from the total volume of cell culture media added.

For example, if seeding a T150, subtract the 4 mL of cells from 30 mL total volume to get 26 mL media.

11.19. Label flasks with the lot number, passage number, cell concentration, current date and analyst initials. The first split after thaw is considered P1. See Attachment 2 for flask label.

11.20. Incubate the flasks in a 37°C, 5% CO_2 incubator for 3-4 days until 70-95% confluent. Do not overgrow.

11.21. Record flasks on HSL_LAB_001.03.

11.22. Repeat steps 11.1 to 11.24 to culture cells to desired passage. Proceed to section 12 if freezing cells.

12. FREEZING OF 293TT CELLS

Note: It is important to chill all components needed to freeze down the cells prior to starting.

12.1. Chill a rack with labeled cryotubes to 2-8ºC. See Attachment 2 for vial label.

12.2. Chill the Freezing Media (293TT FM) on wet ice inside the BSC.

12.3. Chill the CoolCell device to 2-8ºC.

12.4. Remove the flasks from the 37°C, 5% CO_2 incubator and place into BSC.

Note: Only utilize 293TT cells which have grown to 70-95% confluency.

12.5. Remove media from flasks and process per the following steps.

12.5.1. Place the flask upright and tilt it so that media collects into a corner of the side of the flask which does not contain cells to minimize loss of cells.

12.5.2. Using a sterile serological pipette, aspirate the media from the flask and transfer into a 50 mL conical tube (termed “conditioned media”).

12.5.3. Centrifuge the conditioned media at 300 x g, 4°C, 5 minutes per “HSL_EQ_003: Use and Maintenance of the Thermo Fisher Sorvall Legend XTR Centrifuge in the HPV Serology Laboratory.”

12.5.4. Transfer the conditioned media to a new 50 mL conical tube avoiding the cell debris at the bottom of the tube.

12.6. Using a sterile serological pipette, add volume of sterile DPBS per Table 1 to the side of each flask. Do not dispense DPBS directly onto cells.

12.7. Gently rinse cells with DPBS by slowly rotating the flask, so that DPBS washes over the cells. Rotate flask 3-5 times and then stand flask upright again.

12.8. Discard DPBS into waste container.

12.8.1. Tilt flask so the DPBS collects into a corner of the flask where there are no cells adhering to flask surface.

12.8.2. Aspirate DPBS from the flask and discard into the waste container using a sterile serological pipette.

12.9. Wash flasks one additional time per steps 12.6 to 12.8.

12.10. Using a sterile serological pipette, add 0.05% Trypsin-EDTA into each flask as defined in Table 2.

12.11. Gently rotate the flask to distribute the trypsin evenly over the cells.

12.12. Place the flask(s) into a 37°C, 5% CO_2 incubator for 3-5 minutes. Use a timer during this step to minimize time cells are exposed to the trypsin, since prolonged exposure is toxic to cells.

Note: Optimal incubation temperature for trypsin is 37°C. Analyst writes comment on HSL_LAB_001.03 if another incubation temperature used (e.g., Room Temperature).

12.13. After the incubation, take the flasks out and rock back and forth to detach the cells completely or gently tap flask until all cells have detached. Verify cells have detached with an inverted microscope per HSL_EQ_018.

12.14. Using a sterile serological pipette, add 293TT MM to each flask as defined in Table 3 and gently rinse the flask with the media.

12.15. Using a sterile serological pipette, remove media and cells from the flask and transfer into a 50 mL conical tube.

12.16. Add additional 293TT MM to the conical tube to bring volume up to 40-50 mL.

12.17. Tighten cap and gently mix the cells by inverting the tube several times.

12.18. Count cells per HSL_EQ_018 or Attachment 1.

12.19. Capture cell counts and viability on “HSL_LAB_001.04: 293TT Cell Culture Freezing Form.”

Note: Record cell count to the nearest tenth.

12.19.1. Viability for each count must be ≥ 80% for cell concentration to be used. If the viability fails, the cell count is not used to calculate cell concentration. Repeat steps 12.18 and 12.19 as needed; refer to Table 4.

12.19.2. The Percent Difference of passing Cell Concentration 1 and Cell Concentration 2 needs to be ≤ 25% for the count to be considered valid. See step 11.17.2.1 for Percent Difference calculation. If the results are not ≤ 25%, repeat steps 12.18 and 12.19 as needed; refer to Table 4.

Note: Record Percent Difference as a whole number.

12.20. Centrifuge the 293TT cells in the 50 mL conical tube at 300 x g, 20-25°C for 5 minutes per HSL_EQ_003.

12.21. Decant the supernatant and suspend the cells at $10x10^6$ cells/mL using the conditioned media harvested in step 11.5.

12.22. Chill conical tube with cells on wet ice inside BSC.

12.23. Add equal volume of chilled 293TT FM, making a 1:2 ratio (e.g., 10 mL of 293TT cells + 10 mL of 293TT FM), for a final concentration of $5x10^6$ cells/mL.

12.24. Aliquot 1.0 mL culture into each chilled cryotube.

12.25. For controlled-rate freezing, follow "HSL_EQ_022: Controlled-Rate Cell Freezing Using a CoolCell Device."

12.26. After the control-rate freezing procedure, transfer vials into designated box and store in LN_2 freezer. See Attachment 3 for box label and label placement.

12.27. Record final aliquot storage location on HSL_LAB_001.04 and update LN_2 freezer inventory file.

13. ATTACHMENTS

13.1. Attachment 1: Counting Cells with a Hemocytometer

13.2. Attachment 2: 293TT Cell Flask Label and Cryovial Label

13.3. Attachment 3: Box Label Example

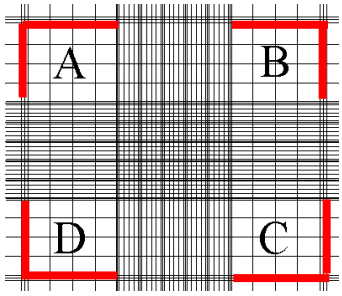
13.4. Attachment 4: HSL_LAB_001.02: 293TT Cell Thaw Form

13.5. Attachment 5: HSL_LAB_001.03: 293TT Cell Culture Maintenance Form

13.6. Attachment 6: HSL_LAB_001.04: 293TT Cell Culture Freezing Form

Attachment 1: Counting Cells with a Hemocytometer

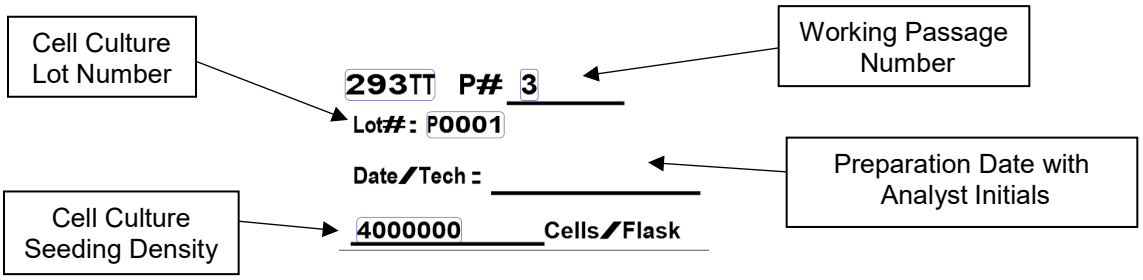
- Count cells with a hemocytometer using trypan blue. Use equal volumes of trypan blue and cells.
- Record the number of live cells (trypan blue negative) and dead cells (trypan blue positive) cells in a QA Issued Laboratory Notebook.
- Refer to Notebook Number and Page Number on assay form and/or logbook forms where counts are used. Analyst is responsible for having notebook entry reviewed by Laboratory Manager.
- To count cells, add 10 µL of trypan blue/cell mixture to hemocytometer.
- Count cells in quadrants A, B, C, and D (refer to diagram below). Approximately 80-200 cells are expected to be present from the combined cell counts from the four quadrants. If significantly different from this, check cell stock or perform a different dilution if needed. Only count cells that fall on two of the four outer edges of the quadrant, as defined by the red lines depicted in the diagram below.



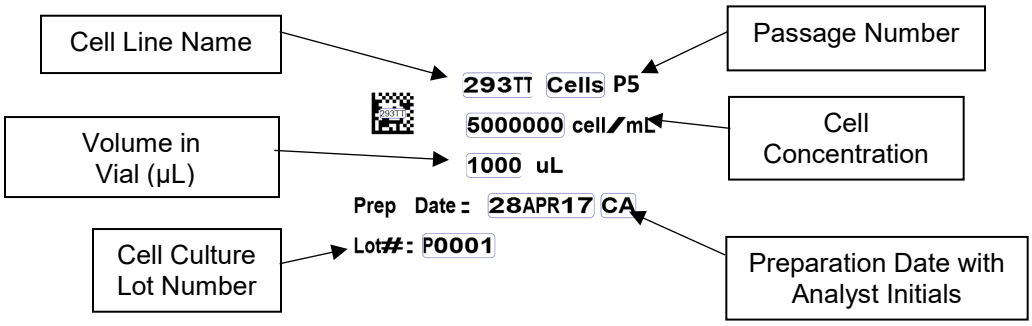
- To calculate cell concentration, take the average of all the cell counts (total cells counted/ # of quadrants counted [A, B, C, and D]). Multiply this number by the dilution factor, then, multiply by 10,000. This will provide cell number per mL.
- For example, if you dilute your sample 1:2 with trypan blue and count 100 live cells in all four quadrants, then the cell concentration would be the following:

(100 ÷ 4) x 2 x 10,000 = 500,000 cells/mL

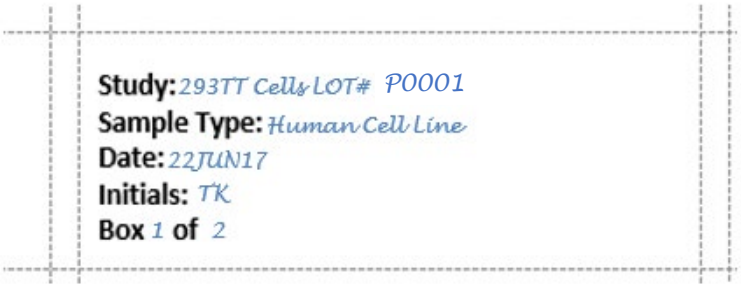
Attachment 2: 293TT Cell Flask Label and Cryovial Label



Note: A template can be used to print labels for the flasks, for ease of labelling. Template can be found at *O:\HSL\HSL_Templates\HSLLabels\HSL_LAB_001_CultureFlask*.



Attachment 3: Box Label Example



Attachment 4: HSL_LAB_001.02: 293TT Cell Thaw Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: 293TT Cell Thaw Form			
Document ID: HSL_LAB_001.02		Version:	5.1
Associated SOP: HSL_LAB_001		Effective Date:	
Supersedes Version:	5.0	**Page 1 of 1**	

Equipment

Equipment Name	Equipment ID	Calibration Due Date
Water Bath	☐ HSL_010 ☐ Other:	☐N/A
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐ Other:	
Cellometer Auto 2000	☐ HSL_019 ☐ Other:	☐N/A
CO_2 Incubator	☐HSL_026 ☐HSL_027 ☐HSL_023 ☐HSL_024 ☐ Other:	
Pipette: µL	PIP_	

Reagents

Reagent Name	Lot Number	Expiration Date
Thawing Media (293TT TM)		
Vita Stain AOPI Staining Solution		

Viability Check

Initial Thawed Vial		Repeat Viability Check on new vial if 1st Fails ☐ N/A	
Cell Count 1 (Cells/ mL)	Viability 1 (%) (≥ 60%)	Cell Count 2 (Cells/ mL)	Viability 2 (%) (≥ 60%)
	☐ Pass ☐ Fail		☐ Pass ☐ Fail

Inoculation

Approximate Volume of Cells (mL)	Volume of 293TT TM (mL)	Flask Type / # Prepared

Cell Vial Label Information / Comments: ☐ Freezer Inventory Updated

Performed by/date:	
Reviewed by/date:	

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

Attachment 5: HSL_LAB_001.03: Cell Culture Maintenance Form

Frederick National Laboratory for Cancer Research
sponsored by the National Cancer Institute

HPV Serology Laboratory
Standard Operating Procedure
Form

Form Title: 293TT Cell Culture Maintenance Form

Document ID: HSL_LAB_001.03		Version:	5.1
Associated SOP: HSL_LAB_001		Effective Date:	
Supersedes Version:	5.0	**Page 1 of 2**	

Cell Culture Maintenance

Working Passage #: _____ to Generated Passage #: _____

Equipment

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐ Other:	
Inverted Microscope	☐ HSL_020 ☐ Other:	☐ N/A
Cellometer Auto 2000	☐ HSL_019 ☐ Other:	☐ N/A
$C0_2$ Incubator	☐ HSL_026 ☐ HSL_027 ☐ HSL_023 ☐ HSL_024 ☐ Other:	
Pipette: µL	P|P_	

Reagents

Reagent	Lot Number	Expiration Date
DPBS		
Trypsin-EDTA		
Maintenance Media (293TT MM)		
Vita Stain AOPI Staining Solution		

Cell Flask Confluency: _____%

Cell Count

Count Number	Cell Concentration (x10^6 Cells/mL)	Viability (%) (≥80%)
1		☐ Pass ☐ Fail
2		☐ Pass ☐ Fail
3 ☐ N/A Row		☐ Pass ☐ Fail
4 ☐ N/A Row		☐ Pass ☐ Fail
Average Cell Count (x10^6 Cells/mL)		
Percent Difference (%) (≤ 25%)		
Secondary Average Cell Count (x10^6 Cells/mL) ☐ N/A Row		

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Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: 293TT Cell Culture Maintenance Form			
Document ID: HSL_LAB_001.03		Version:	5.1
Associated SOP: HSL_LAB_001		Effective Date:	
Supersedes Version:	5.0	**Page 2 of 2**	

Flask Inoculation

Seeding Conc. of Flask ($x10^6$ Cells / Flask)	Total Volume Required (mL / Flask)	Volume of Cells (mL / Flask)	Volume of 293TT MM (mL / Flask)	Flask Type / # Prepared
☐ N/A Row				
☐ N/A Row				
☐ N/A Row				
☐ N/A Row				

Comments:

☐ N/A

Performed by/date:	
Reviewed by/date:	

Attachment 6: HSL_LAB_001.04: 293TT Cell Culture Freezing Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		HPV Serology Laboratory Standard Operating Procedure Form	
Form Title: 293TT Cell Culture Freezing Form			
Document ID: HSL_LAB_001.04		Version:	5.1
Associated SOP: HSL_LAB_001		Effective Date:	
Supersedes Version:	5.0	**Page 1 of 1**	

Equipment

Equipment Name	Equipment ID	Calibration Due Date
BSC	☐ HSL_007 ☐ HSL_008 ☐ HSL_009 ☐ Other:	
Centrifuge	☐ HSL_033 ☐ Other:	
Pipette: µL	PIP_	
☐ N/A Pipette: µL	PIP_	
☐ N/A -80°C Freezer	☐ HSL_022 ☐ HSL_052 ☐ Other:	
LN_2 Tank	☐ HSL_028 ☐ Other:	
	☐ LN_2 Freezer Inventory Updated	
	Rack #:	**Position:**

Reagents

Reagent Name	Lot Number	Expiration Date
Freezing Media (293TT FM)		

Cell Reference

Lot Number	Date of Passage	Passage # Used	Sample of Final Aliquot Label

Cell Suspension

Cell concentration (Cells/mL)	Volume of 293TT FM (mL)	Volume of Cells (mL)

Total Number of Aliquots Prepared: _____

Comments: ☐ N/A

Performed by/date:	
Reviewed by/date:	

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